

What is claimed is

Claim 1) A Method for production of multiple Class I human leukocyte antigens comprising;

a) isolating a mammalian DNA specimen,

b) using a PCR reaction with a pair of flanking oligonucleotide primers and truncating primers amplify a first segment of said DNA specimen that codes for Class I of a major histocompatibility complex (MHC) gene , and truncates said Class I by removal of those regions that encode transmembrane and cytoplasmic domains of said class I MHC molecules;

c) sequencing a DNA product from b) to confirm the identity and fidelity of said truncated molecules, (an automatic sequencer may be used)

d) taking said truncated molecule and cloning it into a mammalian expression vector, and growing by use of normal replication means,

e) transfecting said expression vector with said truncated Class I molecule into a human immortalised cell line,

f) growing product from e) and screening for most active HLA producing cells by limiting dilution and ELISA assay and starting from one molecule growing cells of said good producers by conventional tissue culture methods, harvesting and resuspending in fresh media; and inoculating a hollow fiber reactor unit with said media;

g) feeding said fresh media into said hollow fiber bioreactor unit for continual quantity production of secreted Class I HLA cells into which said Class truncated molecule has been transfected;

i) producing multiple other HLAs by transfecting other Class I alleles into said immortalised cell line and repeating steps b) through g).

Claim 2) A method for production of multiple Class I human leukocyte antigens as in Claim 1)wherein said fresh media was fed into said hollow fiber bioreactor units at a rate to maintain optimum cell growth with oxygen and glucose levels delivered to said cells in said fresh media with harvest rates maintained at a desired level of soluble Class I antigens.

Claim 3) A Method for production of multiple Class I human leukocyte antigens comprising;

a) isolating a mammalian DNA specimen,
b) using a PCR reaction with a pair of oligonucleotide flanking primers and a primer to truncate and add a tail , amplifying a first segment of said DNA specimen that codes for Class I of a major histocompatibility complex (MHC) gene, and adds said tail and truncates said Class I by removal of those regions that encode transmembrane and cytoplasmic domains of said class I

MHC molecules;

- c) sequencing a product from b) to confirm the identity and fidelity of said truncated molecules, (an automatic sequencer may be used)
- d) taking said truncated molecule and cloning said molecule into a mammalian expression vector, and growing by use of normal replication means,
- e) transfecting said expression vector with said truncated Class I molecule into a mammalian immortalised cell line,
- f) growing product from e) and screening for most active HLA producing cells by limiting dilution and ELISA assay and starting from one cell growing cells of said good producers by conventional tissue culture methods, harvesting and pelleting said cells and resuspending in fresh media,
- g) feeding said fresh media into hollow fiber bioreactor units for continual quantity production-of-secreted Class I HLA cells from which said Class I truncated molecule has been transfected;
- i) producing multiple other HLAs by transfecting other Class I alleles into said immortalised cell line and repeating steps b) through g) .

Claim 4) A method for production of multiple Class I human leukocyte antigens comprising:

- a) starting with an immortalised cell line containing a

segment of DNA encoding MHC(major histocompatibilitycomplex) class I molecules;

b)growing said cell line in growth media; spinning down and extracting total RNA (ribonucleic acid);

c) converting said RNA to cDNA using reverse transcriptase in a first strand cDNA synthesis reaction;

d) performing PCR on said cDNA using thermostable polymerase and primer means to add tails and truncate a sequence that encodes cytoplasmic and transmembrane domains, thereby causing said truncated cells to secrete Class I antigens;

f) purifying said truncated PCR product using known purification means;

g)using restriction enzymes cut both strands of said truncated PCR product at specific sequence sites;..

h) using T4 ligase enzyme insert said truncated PCR product into a mammalian expression vector;

i) transforming a bacterial strain by causing said ligated vector to enter said bacteria;

j) plating out said transformed bacterial strain on bacterial media plates containing a suitable antibiotic to enable selection of said of said transformed bacteria and growing overnight ;colonies that grow should be antibiotic resistant,

k) choosing said antibiotic resistant colonies and growing in a suitable liquid containing said suitable antibiotic for

approximately 18 hours while shaking at a suitable speed; and then making glycerol stocks of each;

l) extracting said vector from k) by using a Promega Wizard mini prep kit;

m) starting with product from l) and using restriction enzymes, carry out restriction digest reactions ;run said restriction digest on an ethidium bromide agarose elecrophoresesis gel to confirm the presence of insert;

n) carrying out sequencing reactions and running a sequencing gel to analyze data from said reactions to make certain that said insert has no errors;

o) using a good clone as determined from n) pick a proper one of said glycerol stocks and streak a bacterial media plate containing a suitable antibiotic to select for resistance and grow overnight; picking a colony from said plate, place in 45 mls of liquid media containing said antibiotic and grow for 16 hours while shaking at a suitable speed;

p) extracting said vector containing said PCR product insert from said 45 mls of solution;

q) growing a cell line that does not express Class 1 molecules until said line is in log phase;

.r)inserting said vector containing said PCR product insert into cells in said cell line from q) by known methods;

s) treating said transfected cells with an antibiotic to

choose cells resistant to said antibiotic; said cells that contain said vector with said insert therein are antibiotic resistant;

t) using cells from s) serially dilute said cells in a well plate to a point that there is one cell per well and using ELISA Assay determine which of said cells are producing the most soluble HLA;

u) growing said cells producing the most soluble HLA by conventional tissue culture means ,harvesting and pelletting said cells and resuspending in fresh growth media.;

v) feeding said cells in said fresh media into a hollow fiber bioreactor system counter current to harvest media;

w) feeding oxygen and carbon dioxide into said circulating harvest media and controlling said feed rate ,said oxygen feed, said carbon dioxide feed,said circulating harvest media, and pH and temperature to harvest secreted C lass1 molecules,